United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Veterinary Services

National Veterinary Services Laboratories

National Animal Health Laboratory Network

P.O. Box 844
1920 Dayton Avenue
Ames, IA 50010
(515)337-7731
(515)337-7397 Fax

Assay Performance Characteristics Summary Sheet

Assay: Plum Island Animal Disease Center (PIADC) Classical swine fever virus (CSFV) real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay

Disease: Classical swine fever (CSF)

Agent: Classical swine fever virus (CSFV, Select Agent)

Type of Assay: rRT-PCR assay

Purpose of Assay: Screening [National Animal Health Laboratory Network (NAHLN)], Confirmatory [National Veterinary Services Laboratories (NVSL) reference laboratories]

Background Information: The PIADC CSFV rRT-PCR assay was designed to provide an assay with enhanced performance characteristics [i.e., Tetracore, Inc. (1,2)]. This assay is intended to be used with RNA extracted from diagnostic samples for the detection of CSF viral RNA. The target of the CSF rRT-PCR assay is the 5'UTR region of the CSF genome. This assay was originally developed and validated as a "wet" chemistry assay (using the Qiagen QuantiTect RT-PCR Chemistry) on the Applied Biosystems ABI 7900, 96-well platform. Performance characteristics of this assay were subsequently determined on the ABI 7500 and ABI 7900 using multiple chemistries. The two chemistries demonstrating the best performance characteristics were the Qiagen Multiplex RT-PCR NR and the Invitrogen SuperScript III Platinum One-Step qRT-PCR chemistries.

Platform(s): Applied Biosystems ABI 7900 & ABI 7500

Chemistry(ies):
ABI 7900: Qiagen Multiplex RT-PCR NR Chemistry
ABI 7900: Invitrogen SuperScript™III Platinum® One-Step qRT-PCR Chemistry
ABI 7500: Qiagen Multiplex RT-PCR NR Chemistry
ABI 7500: Invitrogen SuperScript™III Platinum® One-Step qRT-PCR Chemistry

Sample Type: Tissue grown virus, Tonsil, Nasal Swabs and Tonsil Scrapings

Species: Swine

Performance Characteristics: Analytical performance characteristics were determined using a well defined panel of CSF, Borders Disease Virus (BDV) and Bovine Viral Diarrhea (BVD) virus isolates from the Foreign Animal Disease Diagnostic Laboratory repository. In addition, analytical sensitivity and specificity was further characterized utilizing 176 tissue culture grown viruses (CSF, BVD and BDV) obtained from the European Union CSF Reference Laboratory in Hannover, Germany.

Applied Biosystems ABI 7900 & ABI 7500

ABI 7900: "Wet" Applied Biosystems Ez rTth Kit-Limits of Detection Assays	
Analytical Sensitivity-Qiagen Multiplex NR	1-3 $TCID_{50}$/ml
Analytical Sensitivity-Invitrogen SSIII Platinum	< 1-2 $TCID_{50}$/ml
Analytical Specificity	100%

ABI 7500: "Wet" Applied Biosystems-Limits of Detection Assays	
Analytical Sensitivity-Qiagen Multiplex NR	1-2.5 $TCID_{50}$/ml
Analytical Sensitivity-Superscript III Platinum	1-2.5 $TCID_{50}$/ml
Analytical Specificity	100%

Amplification Efficiency: 100% for Qiagen NR Multiplex on ABI 7900
87% for Invitrogen Superscript III Platinum on ABI 7900
97% for Qiagen NR Multiplex on ABI 7500
93% for Invitrogen Superscript III Platinum on ABI 7500

Assay performance on EU CSFV Reference Laboratory Samples (See above for sample description)-A comparison between the PIADC and Tetracore CSFV Assays

Diagnostic Sensitivity and Specificity Based on EU Reference Laboratory Field Samples		
	PIADC-ABI 7900	Tetracore - SmartCycler®
Sensitivity	>0.99 (95% *CI*: 0.96, 1.00)	>0.99 (95% *CI*: 0.99, 1.00)
Specificity	0.92 (95% *CI*: 0.58, 0.99)	0.68 (95% *CI*: 0.40, 0.87)

Repeatability:

Serial variations with a known status sample (Intra-Assay Variability): This experiment was conducted such that an initial sample was used to prepare a 10-fold dilution series from undiluted to 10^{-3}. Once the dilutions were prepared, the RNA was extracted. The RNA resulting from each extraction was run 10 times to generate the data. The samples were prepared in a manner to eliminate any extraneous sources of variation because the goal was to compare intra-platform variability for the different platforms. The table below gives estimates and confidence intervals for the standard deviation associated with residual variance. Again, this is an indication of the amount of variability within a run for these assay/chemistry/platform combinations.

Estimates for Each Platform of the Intra-assay Standard Deviation	
Platform	Estimate (95% *CI*)
PIADC QuantiTect Assay, ABI 7900	0.13 (95% *CI*: 0.10, 0.16)
Tetracore - SmartCycler® Vitrified Assay	1.00 (95% *CI*: 0.81, 1.30)
Tetracore Assay, ABI 7900	0.16 (95% *CI*: 0.13, 0.21)

Diagnostic Performance Characteristics: Diagnostic performance characteristics were evaluated using tonsil samples collected from experimentally infected swine and nasal swabs collected from farms in Colombia and the Dominican Republic (DR). Animals sampled in Colombia were included as part of a negative cohort study. Nasal swab samples from the DR were collected from nine farms in the central region of the DR (1). Animals in the DR are routinely vaccinated with a live attenuated or CSFV E2 subunit vaccine.

Agreement Between Tetracore - SmartCycler® and PIADC - Qiagen Assay on the ABI 7900 using a subset of the Columbian and Dominican Republic Samples		
	PIADC QuantiTect ABI 7900	
Tetracore Cepheid Smart Cycler	Positive	Negative
Positive	54	0
Negative	5	93

Diagnostic Performance Equivalency-ABI 7900 and ABI 7500: Diagnostic equivalency between the ABI 7900 and ABI 7500 platforms was demonstrated utilizing known positive samples from swine experimentally infected at PIADC. Samples from noninfected swine (known negative reference samples) were also utilized for this study. Statistical analysis was performed on the data and platform performance was compared utilizing the correlation coefficient (r) and the concordance correlation coefficient (r_c). This type of data and statistical analysis allowed for comparison of the two different platforms (ABI 7900 and ABI 7500) when utilized with a specific chemistry.

Correlation Coefficients Comparing the ABI 7900 and the ABI 7500 Platforms And Comparing Difference in Mean Ct Values			
Chemistry	r (95% *CI*)	r_c (95% *CI*)	Difference in Means (95% *CI*)
Invitrogen SS III	0.99 (95% *CI*: 0.98, 1.00)	0.88 (95% *CI*: 0.45, 0.94)	2.28 (95% *CI*: 1.91, 2.64)
Qiagen Multiplex	0.99 (95% *CI*: 0.96, 1.00)	0.87 (95% *CI*: 0.53, 0.93)	2.24 (95% *CI*: 1.88, 2.61)

The concordance correlations in the table above suggest that the Ct values of the ABI 7500 plotted against the Ct values obtained from the ABI 7900 do not fall on a 45 degree line. The data shows that the Ct values obtained from the ABI 7500 are lower than those obtained from the ABI 7900 when using either the Invitrogen SS III or Qiagen Multiplex chemistries.

Diagnostic Performance Equivalency-Chemistries on Respective platforms: Diagnostic equivalency between the Invitrogen Superscript III and the Qiagen Multiplex Kit was demonstrated utilizing known positive samples from swine experimentally infected at PIADC. Samples from noninfected swine (known negative reference samples) were also utilized for this study. Statistical analysis was performed on the data and platform performance is compared utilizing the correlation coefficient (r) and the concordance correlation coefficient (r_c). This type of data and statistical analysis allowed for comparison of the two different chemistries on each of the Applied Biosystems platforms (ABI 7900 and ABI 7500).

Correlation Coefficients Comparing the Chemistries on the ABI 7900 and ABI 7500 And Comparing Differences in Mean Ct values			
Chemistries	r (95% *CI*)	r_c (95% *CI*)	Difference in Means (95% *CI*)
Invitrogen SSIII & Qiagen Multiplex-ABI 7500	0.99 (95% *CI*: 0.95, 1.00)	0.91 (95% *CI*: 0.60, 0.97)	1.84 (95% *CI*: 1.47, 2.20)
Invitrogen SSIII & Qiagen Multiplex-ABI 7900	0.99 (95% *CI*: 0.97, 1.00)	0.90 (95% *CI*: 0.62, 0.96)	1.87 (95% *CI*: 1.50, 2.24)

The table above indicates that the Invitrogen SSIII chemistry produces a lower Ct value than the Qiagen Multiplex chemistry.

References:

1. Risatti G, Holinka L, Lu Z, Kutish G, Callahan JD, Nelson WM, Brea Tio E, and Borca MV. 2005. Diagnostic evaluation of a real-time reverse transcriptase PCR assay for detection of classical swine fever virus. *J Clin Microbiol* 43:468-471.
2. Risatti GR, Callahan JD, Nelson WM, and Borca MV. 2003. Rapid detection of classical swine fever virus by a portable real-time reverse transcriptase PCR assay. *J Clin Microbiol* 41:500-505.